

PRELIMINARY AMENDMENT

Serial Number: 09/574,460

Filing Date: May 18, 2000

Title: PRODUCTION OF COMPLEX CARBOHYDRATES

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CLEAN VERSION OF PENDING CLAIMS

6. (Twice Amended) The process of claim 12, wherein the bacteria are *Escherichia coli* K-12 strain JM 109.
7. (Twice Amended) The process of claim 11, wherein the acceptor molecule is N-acetylglucosamine.
8. (Twice Amended) The process of claim 11, wherein the *rfe* gene is from *Haemophilus influenzae*.
11. (Twice Amended) A process for the production of a *Haemophilus influenzae*-specific lipooligosaccharide (LOS) which comprises the steps of:
 - (a) growing in a culture medium gram-negative bacteria comprising (i) a core lipid structure containing a terminal heptose and (ii) a DNA sequence comprising an *rfe* gene, and (iii) an isolated DNA sequence comprising a lipooligosaccharide-synthesis gene (*lsg*) from *Haemophilus influenzae*, wherein the protein encoded by the *rfe* gene is expressed and adds an acceptor molecule to the heptose molecule to synthesize an oligosaccharide; and
 - (b) recovering the *H. influenzae*-specific LOS from the culture medium.
12. (Twice Amended) The process of claim 11 wherein the bacteria are *Escherichia coli*.
18. (Once Amended) The process of claim 11, wherein the DNA sequence comprising an *rfe* gene is part of the gram-negative bacterial genome.
19. (Once Amended) The process of claim 11, wherein the isolated DNA sequence comprising the *lsg* is contained in a vector.
20. (Once Amended) A method of modifying a terminal heptose of a lipopolysaccharide

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(LPS) or lipooligosaccharide (LOS) core structure of a gram-negative bacterial species containing an *rfe* gene comprising regulating the protein encoded by the *rfe* gene with a protein encoded by an isolated *lsgG* gene from *Haemophilus influenzae* in order to catalyze transferring N-acetyl glucosamine onto the terminal heptose.

21. (Once Amended) A process for the production of a complex carbohydrate comprising the steps of:
 - (a) growing in a culture medium gram-negative bacteria comprising (i) a core lipid structure containing a terminal heptose and (ii) a DNA sequence comprising an *rfe* gene, and (iii) an isolated DNA sequence comprising a liposaccharide-synthesis gene G (*lsgG*) from *Haemophilus influenzae*, wherein the protein encoded by the *rfe* gene is expressed and adds an acceptor molecule to the heptose molecule to synthesize complex carbohydrate; and
 - (b) recovering the complex carbohydrate from the culture medium.
22. The process of claim 11, wherein the bacteria are *Salmonella minnesota*.
23. (New) The process of claim 20 or 21, wherein the bacteria are *Escherichia coli*.
24. (New) The process of claim 23, wherein the bacteria are *Escherichia coli* K-12 strain JM 109.
25. (New) The process of claim 21, wherein the acceptor molecule is N-acetylglucosamine.
26. (New) The process of claim 20 or 21, wherein the *rfe* gene is from *Haemophilus influenzae*.
27. (New) The process of claim 20 or 21, wherein the DNA sequence comprising an *rfe* gene is part of the gram-negative bacterial genome.

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28. (New) The process of claim 20 or 21, wherein the isolated DNA sequence comprising the *lsgG* is contained in a vector.

29. (New) The process of claim 20 or 21, wherein the bacteria are *Salmonella minnesota*.